

Biogeography and conservation assessment of *Batrachus* groundwater amphipods (Crangonyctidae) in the central and eastern United States

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Abstract

The subterranean amphipod genus *Batrachus* (Amphipoda: Crangonyctidae) is comprised of eight species that occur in groundwater habitats in karst and glacial deposits of the central and eastern United States. We reexamine the distribution, biogeography, and conservation status of *Batrachus* in light of new species distribution records and divergence time estimates in the genus from a recent molecular study. In particular, we discuss hypotheses regarding the distribution and dispersal of *B. mucronatus* and *B. brachycaudus* into previously glaciated regions of the Central Lowlands. We also conducted the first IUCN Red List conservation assessments and reassessed global NatureServe conservation ranks for each species. We identified 17 threats associated with increased extinction risk that vary in source, scope, and severity among species, with groundwater pollution being the most significant threat to all species. Our conservation assessments indicate that five of the eight species are at an elevated risk of extinction under IUCN Red List or NatureServe criteria, with one species (*B. cellulanus*) already extinct. However, none of the eight species are considered threatened or endangered by any state or federal agency. Significant knowledge gaps regarding the life history, ecology, and demography of each species exist. Given results of our conservation assessments and available information on threats to populations, we offer recommendations for conservation, management, and future research for each species.

Keywords

IUCN, Red List, NatureServe, glaciation, groundwater pollution, climate change, Teays River, Pleistocene

Introduction

The type species of the subterranean amphipod genus *Batrurur* was originally described (as *Crangonyx mucronatus*) from specimens collected from a well in Normal, McClean County, Illinois, USA by Stephan A. Forbes (Forbes 1876). The description was published shortly before his appointment as the new director of the State Laboratory of Natural History, which would later become the Illinois Natural History Survey, with Forbes serving as its first director (Hays 1980; Mills 1958). Nearly 140 years later, we still have much to learn about *Batrurur*, even beneath the very institution where study of this genus began. Over a century passed before a proper revision of this genus was completed (Koenemann and Holsinger 2001).

At present, *Batrurur* comprises eight described species (Table 1) found in the eastern and central United States, in groundwater habitats including caves, springs, wells, and interstitial spaces in glacial sediments. Seven of the eight species are associated with groundwater habitats in karst terranes. Three species found in the Interior Low Plateau and Appalachian Valley are thought to be isolated, endemic relict species (the *B. wilsoni* group), while the other four species associated with karst terranes occur primarily in the Ozark Plateaus. In contrast, *Batrurur mucronatus* (Forbes, 1876), which is the most widely distributed member of the genus, occurs almost exclusively in non-karst habitats in previously glaciated regions of the Central Lowlands. However, *Batrurur brachycaudus* Hubricht & Mackin, 1940, a species largely associated with karst on either side of the Mississippi River Valley in Illinois and Missouri, also has been collected from previously glaciated regions of central Illinois where Koenemann and Holsinger (2001) reported specimens from drain tile outlets in glacial drift (Montgomery and Sangamon counties, Illinois). Unlike *B. brachycaudus* from most karst populations, specimens from central Illinois exhibit sexual dimorphism in the length of the telson, where males have about 20% longer telsons than females. Rather than a new species, Koenemann and Holsinger (2001) treated the Illinois populations as geographic variants of *B. brachycaudus*. Molecular analyses of the small-subunit rDNA gene also supported conspecific treatment (Englisch and Koenemann 2001).

In the course of ongoing studies of groundwater amphipods in North America, we collected specimens of *Batrurur* from central Illinois, unexpectedly discovering specimens of *B. brachycaudus* significantly northeast of its known distribution in central Illinois. Here, we reexamine the distribution and biogeography of *Batrurur* in light of the discovery of this new population, the recent description of a new species from the Ozark Plateaus of Arkansas (Holsinger et al. 2006), and the results of a recent divergence time analysis of amphipods that included three species of *Batrurur* (Corrigan et al. 2014). In particular, we explore hypotheses regarding the distribution and dispersal of *B. mucronatus* and *B. brachycaudus* into previously glaciated regions of the Central Lowlands. Finally, we reviewed threats and conducted the first IUCN Red List conservation assessments for each of the eight species of *Batrurur* and reassess the NatureServe global and state conservation ranks. No *Batrurur* species have been subject to an IUCN Red List conservation status assessment, and NatureServe global conservation status for *Batrurur* species have not been reviewed in 9–14 years (since 2002–2007).

Table 1. The described species of the genus *Bactrurus* (Amphipoda: Crangonyctidae), number of occurrences, type localities, general distribution, and conservation status.

Species	Common Name	Type Locality	States (Counties)	Occurrences	EOO (km ²)	AOO (km ²)	Occurrences on Protected Land	Overall Threat Impact	Previously Assessed	Previous NatureServe Rank	Calculated NatureServe Rank	IUCN Red List Rank	IUCN Red List Criteria	State T & E Status
<i>Bactrurus angulus</i> Koenemann & Holsinger, 2001	Cumberland Gap Cave Amphipod	TN: Claiborne Co.: Sour Kraut Cave (CB46)	TN (1), VA (1)	3	29	12	1	High	2002	G1	G1	CR	B1ab (i,ii,iii,iv)	SGCN [‡] (VA), Rare Animal List (TN)
<i>Bactrurus brachycaudus</i> Hubricht & Mackin, 1940	Short-Tailed Groundwater Amphipod	MO: St. Louis Co.: Spring on Keifer Creek	IL (12), MO (19)	114	90,012	408	41	Medium	2002	G4	G4	LC		absent [‡] (IL), Species of Concern (MO)
<i>Bactrurus cellulanus</i> Koenemann & Holsinger, 2001	Indiana Groundwater Amphipod [§]	IN: Monroe Co.: seep in basement of Jordan Hall, Indiana University, Bloomington	IN (1)	1	na	4	0	Very High	2003	GX	GX	EX		absent [‡] (IN)
<i>Bactrurus hubrichti</i> Shoemaker, 1945	Kansas Well Amphipod	KS: Shawnee Co.: well at Topeka	KS (8), MO (1), OK (2)	15	90,935	52	3	Medium	2002	G4	G3	LC		absent [‡] (KS), Species of Concern (MO), ODWC-II [‡] (OK)
<i>Bactrurus mucronatus</i> (Forbes, 1876)	Glacial Till Groundwater Amphipod [§]	IL: McClean Co.: well at Normal	IA (3), IL (36), IN (26), MI (2), OH (11)	153	259,076	596	8	Medium	2002	G5	G5	LC		absent [‡] (IA, IL, IN, MI, OH)
<i>Bactrurus pseudomucronatus</i> Koenemann & Holsinger, 2001	Ozark Groundwater Amphipod [§]	AR: Randolph Co.: Mansell Cave	AR (2), MO (4)	20	7,230	80	14	Medium	2004	G2G3	G3	NT		SGCN [‡] (AR), Species of Concern (MO)

Species	Common Name	Type Locality	States (Counties)	Occurrences	EOO (km ²)	AOO (km ²)	Occurrences on Protected Land	Overall Threat Impact	Previously Assessed	Previous NatureServe Rank	Calculated NatureServe Rank	IUCN Red List Rank	IUCN Red List Criteria	State T & E Status
<i>Batrachus speleopolis</i> Holsinger, Sawicki & Graening, 2006	Cave City Groundwater Amphipod	AR: Sharp Co.: Cave City Cave	AR (2)	2	na	8	0	High	2007	G1	G1	VU	D2	absent [¶] (AR)
<i>Batrachus wilsoni</i> Koenemann & Holsinger, 2001	Alabama Groundwater Amphipod [§]	AL: Blount Co.: well in kitchen of residence	AL (1)	1	na	4	0	High	2002	G1G2	G1	VU	D2	absent [¶] (AL)

[‡] SGCN = Species of Greatest Conservation Need.

[§] New common name – where prior common names have not been used or accepted, we propose common names to facilitate better communication for conservation and management awareness.

[¶] absent = absent from state list.

[#] ODWC-II = Oklahoma Department of Wildlife Conservation tier status II.

na = not enough occurrences to calculate a minimum convex hull.

Methods

Field surveys

Amphipods were collected from field drain tile outlets and caves by hand and using dip nets. Specimens were transported back to the laboratory alive for photography and subsequent preservation in 80 and 100% ethanol for morphological and future molecular analyses, respectively. Morphological identifications utilized the key and descriptions in Koenemann and Holsinger (2001), original descriptions of species (Forbes 1876; Hubricht and Mackin 1940), and comparisons with museum material.

Compiling distributional records

Distributional data for all *Batrachus* species were compiled from literature sources and biological databases, including Hubricht and Mackin (1940), Hubricht (1943), Barnett (1970), Webb et al. (1998), Koenemann and Holsinger (2001), Reid et al. (2002), Sutton (2003), Holsinger et al. (2006), and Lewis (2015). Additional records from Indiana, collected by Julian Lewis, were obtained from the US National Museum (USNM) and from Lewis (unpublished data, 23 October 2015). Additional records from Illinois were obtained from the Illinois Natural History Survey Crustacean Collection (INHS-CC). Specimen identifications for INHS-CC material were confirmed by microscopic examination (SJT). Distributional data were georeferenced and mapped in ArcMap 10.1 (ESRI 2012) onto the United States karst map (Weary and Doctor 2014). Pleistocene glacial episodes in the central U.S. (Fullerton et al. 2003) also were overlain. Pre-Pleistocene river drainage patterns were adapted from various sources (Teller and Goldthwait 1991; Anderson 1988; Mayden 1988; Cupples and Van Arsdale 2014; Dutch 2015).

Conservation assessments

We conducted conservation assessments under both the IUCN Red List of Threatened Species (<http://www.iucnredlist.org/>) criteria and NatureServe conservation rank protocols (<http://www.natureserve.org/>). In addition to presenting IUCN Red List and NatureServe conservation status ranks, we present conservation status based on state agency listings.

IUCN Red List. Seven IUCN Red List categories are recognized on a continuum of increasing extinction risk (IUCN 2001): Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in the Wild (EW), and Extinct (EX). Two additional categories are also recognized: Data Deficient (DD) in which a species has been evaluated but insufficient data are available to make a determination on conservation rank, and Not Evaluated (NE) in which a

species has yet to be evaluated. The categories Critically Endangered, Endangered, and Vulnerable are considered ‘Threatened’ categories. A species may be classified under one of the ‘Threatened’ categories if it meets specific conditions under any one of five criteria (IUCN 2001): (A) past, present, or projected reduction in population size over three generations; (B) small geographic range in combination with fragmentation, population decline or fluctuations; (C) small population size in combination with decline or fluctuations; (D) very small population or very restricted distribution; or (E) a quantitative analysis of extinction risk. Criteria for threat classification under categories A, C, and E require evidence of declining trends in population size. IUCN Red List assessments followed definitions and guidelines outlined in IUCN (2010). We assessed the status of each species using IUCN Red List categories and criteria (IUCN 2001) using RAMAS Red List 3.0 (Akçakaya et al. 2007) to calculate risk categories.

NatureServe. NatureServe conservation status ranks are based on a one to five scale, from most to least at risk of extinction: G1 (Critically Imperiled), G2 (Imperiled), G3 (Vulnerable), G4 (Apparently Secure), and G5 (Secure). Two additional ranks associated with extinction exist: GH (Possibly Extinct) and GX (Presumed Extinct). Status ranks are assessed at three geographic scales: global (G), national (N), and state (S). We present ranks at the global and state scales. NatureServe ranks are based on ten primary factors grouped into three main categories: rarity, trends, and threats (Master et al. 2009). Rarity factors include range extent of occurrence (EOO), area of occupancy (AOO), number of occurrences, number of occurrences with good viability or ecological integrity, population size, and environmental specificity. Trend factors include both short-term and long-term trends in population size, EOO, AOO, number of occurrences, and viability or ecological integrity of occurrences. Threat factors include threat impact and intrinsic vulnerability to threats. Number of protected or managed occurrences and other factors can also be included to assess conservation status.

NatureServe global conservation status assessments for each lineage were calculated using default points and weights with the NatureServe Rank Calculator v3.186 (Faber-Langendoen et al. 2009; available at www.natureserve.org/conservation-tools/conservation-rank-calculator). We assigned a value of “Very Narrow” for environmental specificity for *B. angulus*, *B. cellulanus*, *B. pseudomucronatus*, *B. speleopolis*, and *B. wilsoni*, as these species are primarily known from only a single habitat type. We assigned a value of “Narrow” for environmental specificity for *B. brachycaudus*, *B. hubrichti*, and *B. mucronatus*. These species are known from subterranean habitats but also springs and seeps.

Geographic range size. IUCN Red List and NatureServe conservation assessments use two different measures of geographic range size: EOO (also referred to as range extent) and AOO. We calculated EOO and AOO using the web-based program GeoCAT (Bachman et al. 2011; available at geocat.kew.org). EOO was calculated as a minimum convex hull. A grid size of 2 km (4 km²) was used to estimate AOO (Faber-Langendoen et al. 2009; IUCN 2010).

Abundance. Accurate estimates of population size and abundance and trends through time are rare for invertebrates in conservation assessments, as such data are

particularly difficult to obtain for most species (Cardoso et al. 2011, 2012; Fox et al. 2011; Adriaens et al. 2015). Consequently, many IUCN assessments of invertebrates have used criteria B and D (Cardoso et al. 2012). Estimates of the reduction in AOO have been used as a surrogate for suspected reductions in population abundance under criterion A in some assessments (e.g., Adriaens et al. 2015), based on the assumption that abundance is correlated with the range of a species (Gaston 1994; Cardoso et al. 2011). However, this approach also has shortcomings (discussed in Cardoso et al. 2011). Abundance data are particularly difficult to obtain for subterranean taxa, because of the inaccessibility of and challenges associated with sampling subterranean habitats. Unsurprisingly, abundance data are limited for most populations of *Batrachus*. However, historical and contemporary abundance data do exist for select populations, particularly those of range restricted species (i.e., *B. angulus*, *B. cellulanus*, and *B. wilsoni*). These data were included when applicable in NatureServe and IUCN Red List assessments.

Trends. The change in EOO, AOO, number of occurrences, and quality of habitat were determined over short- and long-term timescales when such data were available. Trends in abundance over time were largely not incorporated in assessments for reasons mentioned above. Long-term trends were considered from the year of first discovery of a species to the present day, while short-term trends were considered over the last 10 years.

Threats. We followed the threat assessment protocol outlined in Master et al. (2009) and the IUCN-Conservation Measures Partnership Classification of Threats (Salafsky et al. 2008) to evaluate the scope, severity and timing of observed, inferred, and suspected threats to each species. To assist in the identification of current and potential threats, we examined land cover and human population density surrounding occurrences for each species. Land cover data from the 2011 release of the National Land Cover Database (NLCD; Homer et al. 2015) were analyzed around a 2.5 km buffer (19.6 km² area) around each occurrence in ArcGIS v10.3. Because many of these cover types occur only in Alaska or coastal areas, we analyzed 15 major cover types that were collapsed into six broad categories: (1) developed land – open space, low-intensity, medium-intensity, and high-intensity urbanization; (2) forest – deciduous forest, evergreen forest, and mixed forest; (3) grassland/shrub – grassland with >80% total herbaceous vegetation and shrub/scrub; (4) pasture/hay planted for livestock grazing or the production of seed or hay crops; (5) cultivated crops, orchards, and vineyards; and (6) water, including open water and wetlands. We also examined human population density surrounding occurrences as a proxy for urbanization. Human population data were obtained for the 2010 U.S. Census from the U.S. Census Bureau (TIGER/Line® shapefiles available at <https://www.census.gov/geo/maps-data/data/tiger-data.html>). We used the Intersect geoprocessing tool with the buffer shapefile generated previously and the polygon population and housing unit shapefile as input to create a shapefile used to calculate total population and population density within the buffer area around each occurrence. The Dissolve data management tool was used to summarize total population.

Protected and managed occurrences. Although the number of protected or managed occurrences is no longer considered in NatureServe conservation assessments, this information is of value for developing and prioritizing management decisions. We determined whether occurrences for each species occurred on state or federal protected areas (e.g., state parks, natural areas, national parks, state and national forests, etc.). Protected areas were obtained from the USGS Protected Areas Database (PAD-US) version 1.3 (shapefiles available at <http://gapanalysis.usgs.gov/padus/>).

Uncertainty. Uncertainty in values of assessment criteria is an important consideration when assessing conservation status, as uncertainty can strongly influence the assessment of extinction risk (Akçakaya et al. 2000; IUCN 2001; Gillespie et al. 2011). NatureServe accounts for uncertainty by allowing a range of ranks to show the degree of uncertainty in a conservation status when available information does not permit a single status rank (Master et al. 2009). The IUCN Red List assessment also deals with uncertainty by allowing a plausible range of values to be used to evaluate criteria (IUCN 2001, 2010; Mace et al. 2008). For both assessments, we adopted a moderate dispute tolerance considering the most likely plausible range of values for a criterion and excluding extreme or very unlikely values (Faber-Langendoen et al. 2009; IUCN 2010). RAMAS Red List allows the specification of user's attitude to risk and uncertainty by setting values for risk tolerance and dispute tolerance (Akçakaya et al. 2000). Risk tolerance ranges from 0 (extremely precautionary) to 1 (extremely evidentiary), while dispute tolerance ranges from 0 (inclusion of all estimates) to 1 (inclusion of only the consensus estimates). For all assessments, we set risk tolerance to 0.5 (risk neutral) and dispute tolerance to 0.5.

Results

New distribution records

In 2011, David J. Soucek (Illinois Natural History Survey, University of Illinois), collected specimens identified as *B. brachycaudus* from the outlet of a field tile draining into the East Fork Embarras River in Champaign County, Illinois, USA (39.94505°N, 88.12310°W; 23 March 2011; Figs 1, 2, arrow A), and additional material of the same species was later collected from the same field tile outlet in 2015 (MLN & SJT; 28 May 2015). Also on 28 May 2015, we made a collection of *B. mucronatus* in the same county from a historical locality (large concrete drain pipe flowing into drainage ditch, 5.1 km N of Mayview in the Vermilion River Basin; 40.15754°N, 88.10506°W) as was collected by Koenemann and Englisch on 13 May 1999 and identified as *B. mucronatus* by Koenemann and Holsinger (2001). The East Fork Embarras River record (Figs 1, 2, arrow A) represents a 129 km northeastern range extension for *B. brachycaudus*. The distribution of *B. mucronatus* is extended to the southwest in Indiana on the basis of USNM records, and northwards in Illinois to Cook and Lee counties based on INHS-CC records. Finally,

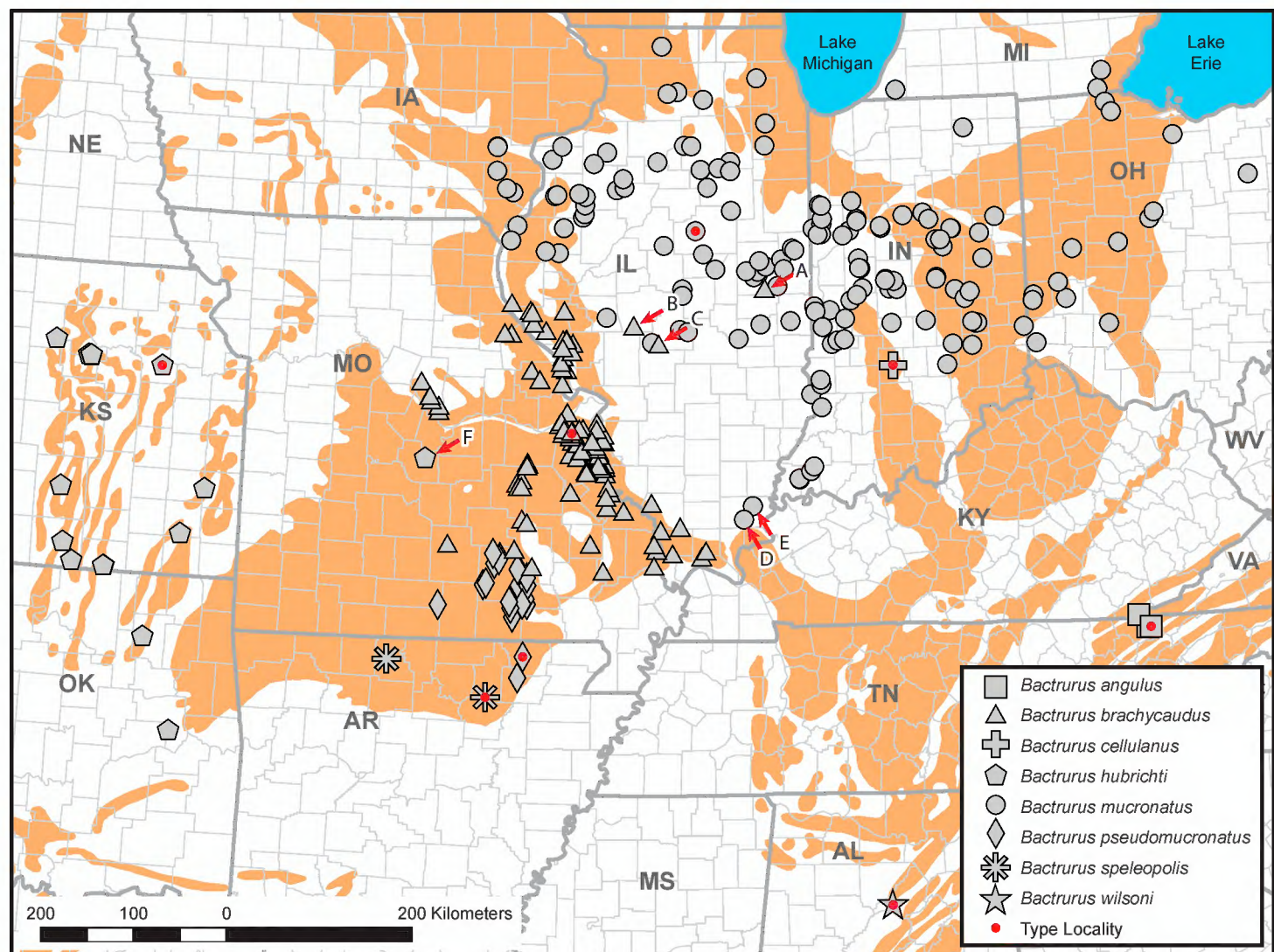


Figure 1. Distribution of species of the genus *Batrurus* (Amphipoda, Crangonyctidae) in relation to karst. Karst areas are based on Weary and Doctor (2014). Letters **A–F** (red arrows) are localities discussed in the body of the text.

we (MLN, SJT, D. Soares, and T. Haspel-Soares) collected a single adult *B. hubrichti* on 13 July 2015 from Klug’s Cave, Miller Co., Missouri.

Distribution and biogeography

Almost all *Batrurus* species are associated with karst terranes (Fig. 1), with the majority of species restricted to a single karst region. Three species, *B. angulus*, *B. cellulanus*, and *B. wilsoni*, have extremely restricted distributions and are endemic to isolated karst areas in the Interior Low Plateau and Appalachian Ridge and Valley. *Batrurus speleopolis* also has a limited distribution but is parapatric to *B. pseudomucronatus* in the Ozark Highlands. Among the broadly distributed species, *B. hubrichti* is associated with the Flint Hills karst in Kansas and Oklahoma (with one notable exception in Missouri: Figs 1, 2, arrow F). *Batrurus brachycaudus* is associated with springs and caves along either side of the Mississippi River. However, three localities in central Illinois (Figs 1, 2, arrows A, B, and C) are disjunct from the main distribution in areas strongly impacted by Pleistocene glacial drift, including the new locality reported above.

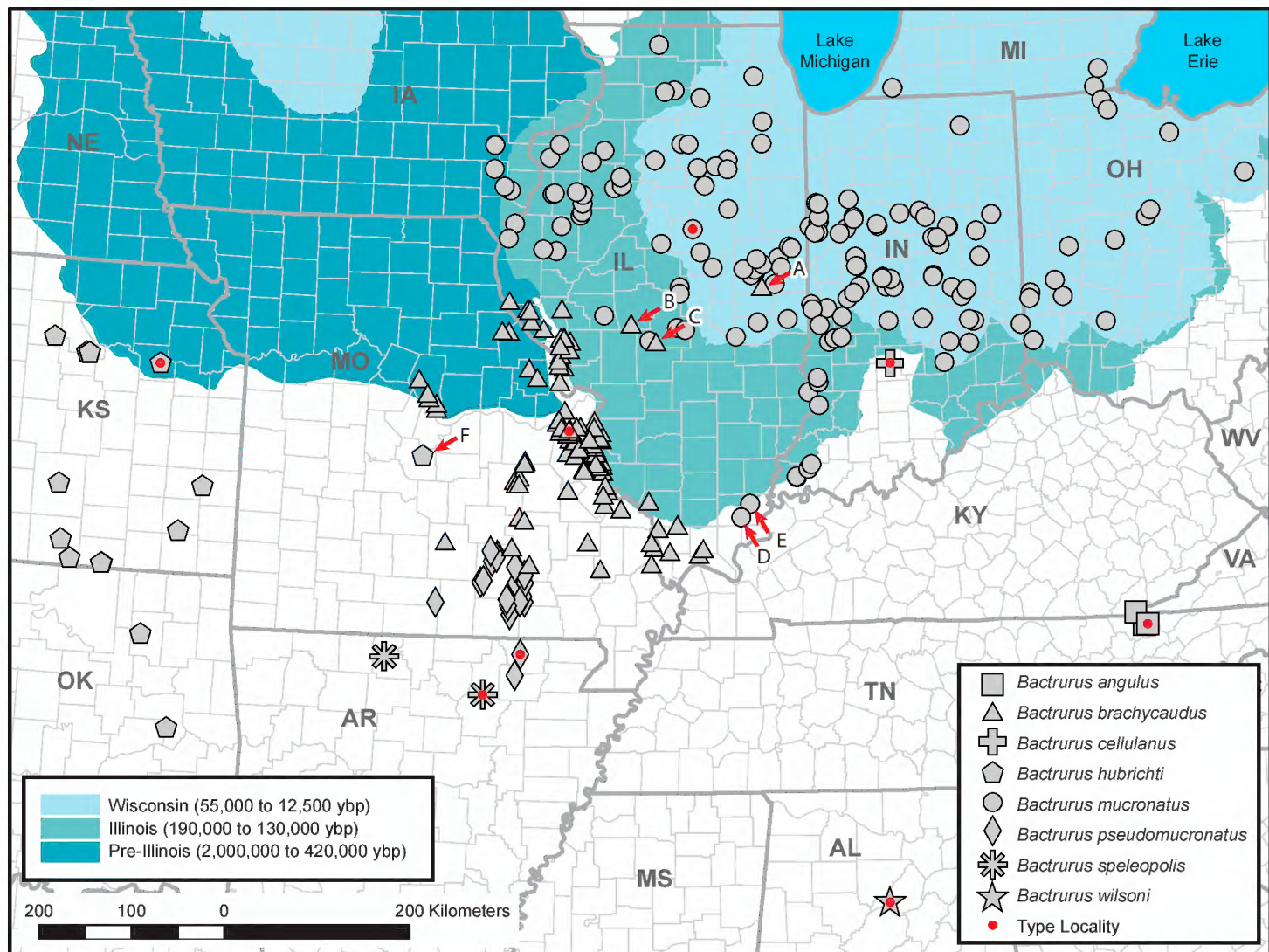


Figure 2. Distribution of species of the genus *Bactrurus* (Amphipoda, Crangonyctidae), overlain on a map of the maximum extent of Pleistocene glacial episodes derived from Fullerton et al. (2003). Letters **A–F** (red arrows) are localities discussed in the body of the text.

Occurrences, EOO, and AOO

We compiled 309 occurrence records from several literature sources, state and personal biological databases, and museum collections for the eight described *Bactrurus* species. The vast majority (86.4%) of these records are associated with two species (Table 1): *B. brachycaudus* and *B. mucronatus*. Two species, *B. cellulanus* and *B. wilsoni*, are known from only a single locality, and two others, *B. angulus* and *B. speleopolis*, are known from three and two localities, respectively. EOO was quite variable among species (Table 1), ranging from 29 km² to 259,076 km² with a mean of $89,456 \pm 104,321$ km². EOO could not be calculated for three species known from two or fewer occurrences: *B. cellulanus*, *B. speleopolis*, and *B. wilsoni*. AOO averaged 146 ± 227 km², with a minimum of 4 km² and a maximum of 596 km².

Abundance

Less than 15 individuals in total have been observed or collected for three species. A total of four specimens of *B. cellulanus* have been collected on three occasions in

1962–1963 from a groundwater seep-stream in the subbasement of Jordan Hall on the campus of Indiana University in Bloomington, Indiana (Koenemann and Holsinger 2001). *Batrachus wilsoni* is known from only one site and six specimens: four specimens were collected from a residential well in Blount Co., Alabama, on three occasions in 1982–1983. However, a single specimen was collected on two separate occasions in June 1999 (Koenemann and Holsinger 2001). The type locality of *B. wilsoni* has not been visited since 1999 to our knowledge. Seven specimens of *B. angulus* were collected from three localities in Claiborne Co., Tennessee, and Lee Co., Virginia, in the 1970s. Repeat visits to these caves in 1996–1997 did not yield any additional observations or specimens (Koenemann and Holsinger 2001). However, six individuals were observed at one locality in Tennessee in June 2015, confirming the continued presence of this species (MLN, unpublished data). Up to 25 individuals have been observed at the type locality of *B. speleopolis* in Sharp Co., Arkansas during surveys in 2001–2004 (Graening et al. 2005; Holsinger et al. 2006).

The other four *Batrachus* species are comparatively more abundant. Although fewer than 10 individuals have been reported during surveys for the majority of occurrences, all four species can be locally abundant. For example, over 30 individuals have been observed during single surveys at one locality of *B. hubrichti*, two localities of *B. pseudomucronatus*, five localities of *B. brachycaudus*, and 13 localities of *B. mucronatus*. Over 1,000 individuals of *B. hubrichti* were reported over a 20-month period in 1990–1992 during a study of a spring at the Konza Prairie Research Natural Area in Riley Co., Kansas (Edler and Dodds 1996).

Trends

EOO and AOO for each *Batrachus* species, with the exception of *B. cellulanus* and *B. angulus*, likely have not decreased significantly in the last 10 or even 50 years. *Batrachus cellulanus* has not been seen since 1963, as termiticide applications to the grounds around the type locality extirpated all aquatic subterranean life, including *B. cellulanus* (Lewis 2012, 2015). *Batrachus angulus* has not been observed from two of the three known occurrences since the 1970s: the type locality in Tennessee since 1977 and from the locality in Virginia since 1979. EOO and AOO have increased for *B. brachycaudus* and *B. mucronatus* in recent years due to increased study and inventory efforts in Illinois and Indiana.

Threats

Dominant land use surrounding occurrences of each species varied widely among species (Fig. 3). Developed (urban) areas was the prevalent land use surrounding occurrences of *B. cellulanus* (86.8% of the area), which occurred in Bloomington, Indiana, but the percentage of developed land was not greater than 15% for any other species.

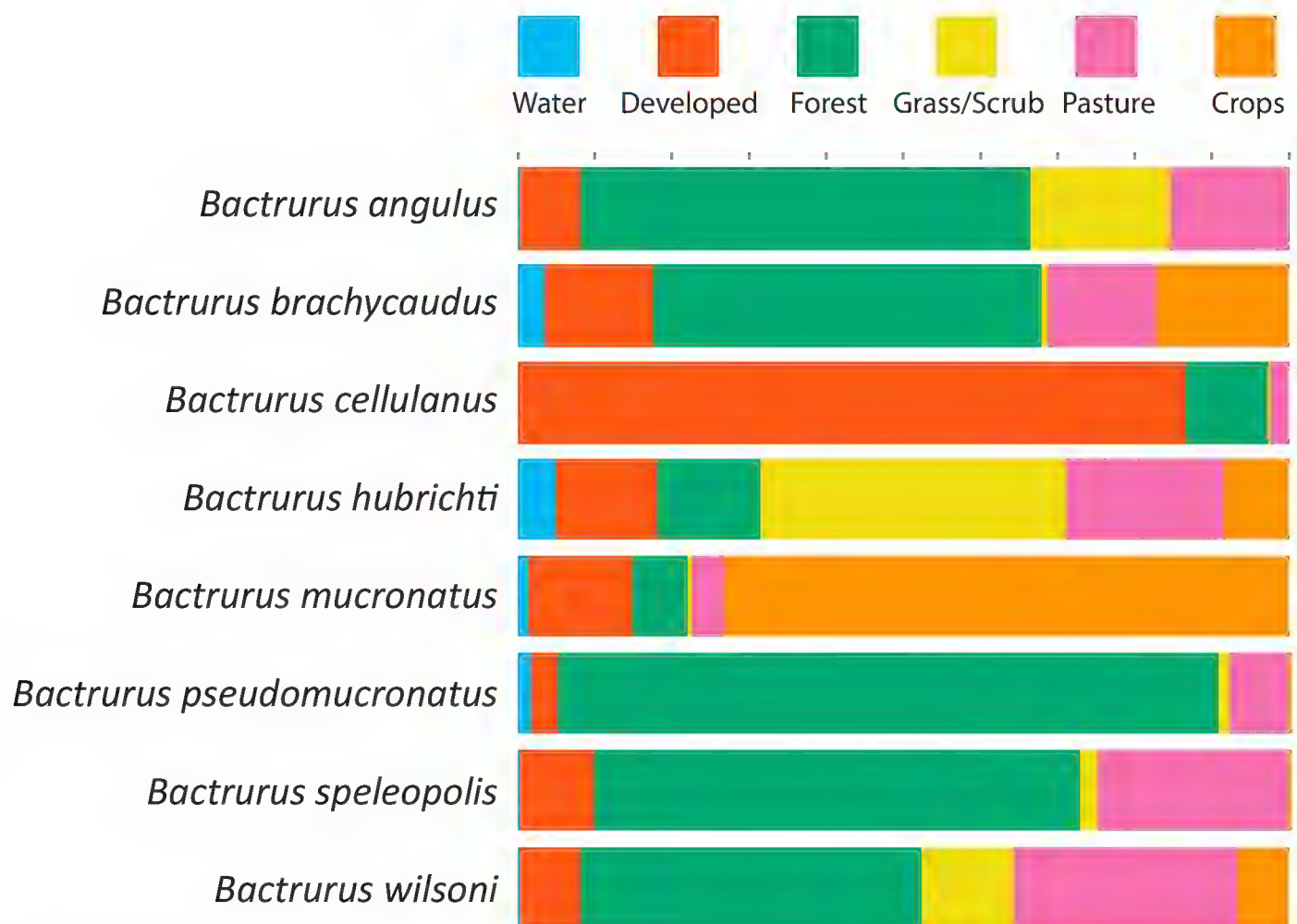


Figure 3. Land use within the ranges of the eight species of the genus *Batrachus*. Based on the National Land Cover Database (Homer et al. 2015), collapsed into six categories (see Methods).

Cropland was the most prevalent land use surrounding occurrences of *B. mucronatus* (73.2%) but was only greater than 10% in one other species (*B. brachycaudus*). Pasture was common (>20%) surrounding occurrences of three species: *B. hubrichti*, *B. speleopolis*, and *B. wilsoni*. Forest was the prevalent land use surrounding occurrences of five species: *B. pseudomucronatus* (85.6%), *B. speleopolis* (62.7%), *B. angulus* (58.3%), *B. brachycaudus* (50.2%), and *B. wilsoni* (44.0%). Grassland-scrub was the prevalent land use surrounding occurrences of *B. hubrichti* (39.8%).

We identified 17 threats that either have been documented or may affect populations of *Batrachus* at present or in the near future (Table 2). These threats vary in impact among *Batrachus* species and likely among regions within the widely distributed species, such as *B. mucronatus*. Groundwater pollution is the primary threat that has the greatest probability of impacting local populations of *Batrachus* species. However, the potential sources of groundwater pollution vary among species depending on land use within the recharge areas of documented occurrences. For example, contamination associated with agriculture, such as pesticides, herbicides, and livestock waste, are more likely to impact populations of *B. mucronatus*, where cropland is the prevailing land use surrounding occurrences. Application of insecticides, herbicides, and fungicides onto lawns, gardens, and landscaping is a threat to populations in urban residential areas. Repeated termiticide application is believed to be the primary cause of extinction of *B. cellulanus* (Lewis 2012, 2015). *Batrachus* populations in urban and rural residen-

Table 2. List of possible threats facing each species of *Batrachus* following the classification proposed by Salafsky et al. (2008). Threat impacts are negligible (N), low (L), medium (M), high (H), and very high (VH).

Threat	<i>B. angulus</i>	<i>B. brachycaudus</i>	<i>B. cellulatus</i>	<i>B. hubrichti</i>	<i>B. mucronatus</i>	<i>B. pseudomucronatus</i>	<i>B. speleopolis</i>	<i>B. wilsoni</i>
1. Residential & commercial development	L	M	H	L	L	L	L	L
1.1. Housing & urban areas	L	L	H	L	L	L	L	L
1.2. Commercial & industrial areas	N	L	M	L	L	N	L	N
2. Agriculture & aquaculture	L	M	L	M	M	L	L	L
2.1. Annual & perennial non-timber crops	N	L	N	L	H	N	N	L
2.3. Livestock farming & ranching	L	L	L	L	L	L	L	L
3. Energy production & mining	N	L	N	N	N	N	N	N
3.1. Oil & gas drilling	N	L	N	N	N	N	N	N
3.2. Mining & quarrying	N	L	N	N	L	N	N	N
4. Transportation & service corridors	L	L	H	L	L	L	L	L
4.1. Roads & railroads	L	L	H	L	L	L	L	L
5. Biological resource use	L	L	L	L	L	L	L	L
5.1. Hunting & collecting animals	L	L	L	L	L	L	L	L
6. Human intrusions & disturbance	L	L	N	L	L	L	L	N
6.1. Recreational activities	L	L	N	L	L	L	L	N
7. Natural system modifications	L	L	N	L	L	L	L	L
7.1. Dams & water management/use	L	L	N	L	L	L	L	L
9. Pollution	L	L	VH	L	M	L	L	L
9.1. Domestic & urban waste water	L	L	VH	L	L	L	L	L
9.2. Industrial & military effluents	N	L	M	L	N	N	N	N
9.3. Agricultural & forestry effluents	L	L	N	L	M	L	L	L
9.4. Garbage & solid waste	N	L	N	N	N	N	L	N
11. Climate change & severe weather	M	L	M	L	L	M	M	M
11.1. Habitat shifting & alteration	M	L	M	L	L	M	M	M
11.2. Droughts	L	L	L	L	L	L	L	L
11.4. Storms & flooding	N	N	N	N	N	N	N	N

tial areas are also susceptible to exposure to leachate from septic tanks and sewers. A growing threat is the increase in conversion of land into impervious surfaces, such as roads, parking lots, and sidewalks, associated with urbanization, particularly for populations of *Batrachus* near growing metropolitan areas.

Any change in land use from natural to disturbed states has the potential to impact populations of *Batrachus* through habitat degradation associated with changes in sediment and nutrient loads, hydrological regimes, and thermal profiles. Other potential threats include mining operations, climate change, over-collection associated the sci-

entific studies, and disturbance of cave populations associated with human visitation for research, recreational, or commercial purposes. At the present time, no evidence exists for a documented decline as a result of these additional threats. The known exception is one population of *B. brachycaudus* was extirpated when a cave system was destroyed by mining in Ste. Genevieve Co., Missouri.

Occurrences on protected areas

The majority of *Batrachus* occurrences are located on private land (78.3%). However, the percentage of protected occurrences (those that occur on state or federal protected land) is highly variable among species. No occurrences of *B. cellulanus*, *B. speleopolis*, and *B. wilsoni* occur on protected lands. In contrast, 70.0% of occurrences of *B. pseudomucronatus* occur on protected land, with most occurring on land units of Mark Twain National Forest or conservation areas owned by the Missouri Department of Conservation. Just 5.2% occurrences of *B. mucronatus* occur on protected land, despite 153 occurrences in total. Between 20.0 and 36.0% of occurrences occur on protected land for the remaining three species (*B. angulus*, *B. brachycaudus*, and *B. hubrichti*).

Conservation status

IUCN Red List. None of the eight species of *Batrachus* had been assessed previously under IUCN Red List criteria (IUCN 2015). Applying IUCN criteria and their associated subcriteria to *Batrachus* species resulted in three species being classified as threatened (Table 1): *B. angulus* as Critically Endangered under criterion B1ab(i,ii,iii,iv), *B. speleopolis* as Vulnerable under criterion D2, and *B. wilsoni* as Vulnerable under criterion D2. *Batrachus cellulanus* was classified as “Extinct.” *Batrachus pseudomucronatus* was classified as “Near Threatened,” as there is no current evidence for a decline in EOO, AOO, or quality of habitat. However, only 20 occurrences are known, and any significant threat could result in the species soon qualifying for “Vulnerable” under criterion B1 or B2. The remaining three species (*B. brachycaudus*, *B. hubrichti*, and *B. mucronatus*) were classified as “Least Concern.”

NatureServe. *Batrachus cellulanus* is “Presumed Extinct” (GX), as this species has not been observed since the 1960s, and additional populations have not been discovered despite intensive surveys of cave and spring communities in karst of southern Indiana. Five of the eight species are considered threatened under NatureServe criteria (Table 1) with a ranking of “Vulnerable” or higher (G1–G3). *Batrachus angulus*, *B. wilsoni*, and *B. speleopolis* are at the highest risk of extinction, with a ranking of “Critically Imperiled” (G1). These three species are known from three or fewer occurrences and have extremely restricted distributions. *Batrachus pseudomucronatus* and *B. hubrichti* were assessed as “Vulnerable” (G3). Only *B. brachycaudus* (G4) and *B. mucronatus* (G5) are not considered at risk of extinction.

Discussion

Biogeography

Of the eight described *Batrachus*, only *B. brachycaudus* and *B. mucronatus* are not entirely associated with karst, although the majority of *B. brachycaudus* localities are known from karst terranes. Only *B. mucronatus* fails by any measure to demonstrate an association with karst terrane, with specimens found broadly across the Midwestern United States from southeastern Iowa to central Ohio and southern Michigan (Fig. 1). Koenemann and Holsinger (2001) discussed the biogeographic distribution of *Batrachus*, noting a concentration of diversity in the Central Lowland and Ozark Plateaus. Only *B. mucronatus* occurs almost exclusively to the north of the maximum extent of glacial ice during the Pleistocene, which is in contrast to spatial patterns of subterranean biodiversity in temperate North America where the vast majority of subterranean biodiversity occurs exclusively south of this line (Culver et al. 2003).

It is often assumed that subterranean habitats were either destroyed or too inhospitable for most subterranean fauna to survive beneath glacial ice sheets during the Pleistocene (Vandel 1965; Peck and Christiansen 1990; Culver and Pipan 2009). Both geological and climatic processes during this epoch have influenced the distributions of temperate subterranean fauna in North America (Barr 1968; Holsinger 2000; Culver and Pipan 2009; Niemiller et al. 2013b). Two primary hypotheses have been proposed to explain the distribution of *B. mucronatus* and other subterranean species that occur north of the line of maximal extent of Pleistocene glacial ice. One possibility is that groundwater amphipods, including *Batrachus*, were able to survive in groundwater refugia beneath the ice (Holsinger 1978, 1981, 1986). Alternatively, amphipods dispersed northward colonizing groundwater in the glacial deposits left behind by the receding glaciers (Holsinger 1978; Lewis and Bowman 1981). Because the closest relative of *B. mucronatus* (*B. pseudomucronatus*) occurs further south in the unglaciated Ozark Plateaus, Holsinger (1986) considered the present-day distribution of *B. mucronatus* more likely reflected the latter scenario. *Batrachus mucronatus* is the only species in the genus not generally restricted to karst, and it is instead adapted to life in saturated soils and underlying layers (Koenemann and Holsinger 2001). Because it is not restricted to karst, *B. mucronatus* was uniquely (unlike most of its congeners) suited to recolonize interstices of loosely consolidated, saturated drift and underlying strata as glaciers retreated, moving into areas formerly covered by the Wisconsin glacial ice sheet only after its retreat. Under this scenario, *B. mucronatus* populations in western and southern Illinois would represent much older populations, established at least 130,000 to 55,000 years ago (i.e., prior to the Illinoian and Wisconsin glacial episodes) and possibly much older (e.g., Miocene, 23.03 to 5.33 mya), while those in central Illinois and areas north and east would represent much younger populations derived from these older, southwestern populations. Under this scenario, the youngest populations of *B. mucronatus* would occur in northern Indiana, northern Ohio, and southern Michigan.

However, Koenemann and Holsinger (2001) suggested post-glacial range expansion may be less likely given the relatively short post-glacial time available for dispersal from the Ozark Plateaus or southwestern Illinois (19,000–10,000 ybp) and the presumed poor dispersal ability of *B. mucronatus*. Koenemann and Holsinger (2001) further argued against a post-glacial range expansion hypothesis by noting that the range of *B. brachycaudus* bisects the collective distributions of *B. mucronatus* and *B. pseudomucronatus*. *Batrachus mucronatus* and *B. pseudomucronatus* are sister species (Koenemann and Holsinger 2001; Corrigan et al. 2014), with the latter occurring in karst terranes of the Ozark Plateaus. Koenemann and Holsinger (2001) hypothesized that *B. mucronatus* originated south of the maximum extent of Pleistocene glaciation, perhaps in southern Illinois and Missouri. As the glaciers receded, divergence of *B. mucronatus* and *B. pseudomucronatus* occurred. In this scenario, relict populations of *B. mucronatus* would be expected to occur between the gap between the distributions of each species that is now occupied by *B. brachycaudus*. Koenemann and Holsinger (2001) hypothesized further that a geographic distributional pattern associated with major post-glacial drainage basins would be expected in *B. mucronatus* under a post-glacial expansion hypothesis; however, such a pattern is notably absent. Koenemann and Holsinger (2001) also suggested that the occurrence of *B. brachycaudus* at several localities north of the maximum extent of glaciation “appear to be exceptional occurrences,” indicating possible barriers to dispersal in glacial sediments in western Illinois. However, our new record 129 km northeast suggests that barriers to dispersal may not be as strong as suggested by Koenemann and Holsinger (2001).

Instead, Koenemann and Holsinger (2001) favored a subglacial refugia hypothesis whereby *B. mucronatus* survived *in situ* under episodic glaciation of the Wisconsin (55,000 to 12,500 ybp) and Illinois (190,000 to 130,000 ybp), moving vertically rather than horizontally into sand and gravel layers deposited by glaciers. While Koenemann and Holsinger (2001) stated that permafrost did not penetrate the ground deeper than 5 meters, allowing persistence of *B. mucronatus* in aqueous media below this level, it is possible that the Pleistocene glaciers were largely free of an underlying permafrost layer because the weight of the glaciers lowers the melting point of water, and temperate glaciers typically have subglacial flowing waters (Sharp 1988). Moreover, there is growing evidence that groundwater amphipods have persisted beneath glacial ice. At least six species of amphipods are thought to have persisted in subglacial refugia. *Stygobromus canadensis* occurs in a section of Castleguard Cave overlain by Mount Castleguard glaciers and the Columbian Icefields in Alberta, Canada (Holsinger 1980, 1981; Holsinger et al. 1983). Three other stygobromids occur in previously glaciated regions and are hypothesized to have persisted in subglacial refugia during the Pleistocene: *S. iowae* (Peck and Christiansen 1990), *S. putealis* (Peck and Christiansen 1990), and *S. tenuis* (Smith 1985). A species and family of Crangonyctoidea (family Crymostygidae), *Crymostygius thingvallensis* Kristjánsson & Svavarsson, 2004 from Iceland, and another crangonyctid, *Crangonyx islandicus* Svavarsson & Kristjánsson, 2006, also from Iceland, are thought to have persisted in subglacial refugia since the Pliocene when glaciers covered Iceland, from 2.6 mya until about 10,000–12,000 ybp (Kristjánsson

and Svavarsson 2004, 2007; Svavarsson and Kristjánsson 2006; Kornobis et al. 2010, 2011). Groundwater amphipods in the genus *Niphargus* (family Niphargidae) likely persisted beneath glaciers during the Pleistocene in northern Europe (McInerney et al. 2014). Finally, experiments by Espinasa et al. (2015) demonstrate that the crangonyctid *Stygobromus allegheniensis* Holsinger, 1967 in the eastern United States can survive being frozen in ice, at least for a short duration.

A recent molecular study by Corrigan et al. (2014) sheds further light on the biogeography of *Batrurur*. This study reconstructed the time-calibrated phylogeny of deep sea amphipods in North America using five loci but also included three species of *Batrurur* as outgroup taxa: *B. brachycaudus*, *B. mucronatus*, and *B. pseudomucronatus* (Fig. 4). Divergence between *B. mucronatus* and *B. pseudomucronatus* was dated at 12–27 mya, while divergence between *B. brachycaudus* and *B. mucronatus* + *B. pseudomucronatus* was dated at 30–58 mya (Corrigan et al. 2014), well before glacial episodes of the Pleistocene. These three lineages were likely well established before the Pleistocene, and, consequently, divergence between *B. mucronatus* and *B. pseudomucronatus* was not driven or associated with Pleistocene glaciation, as suggested by Koenenmann and Holsinger (2001).

We find further support for the subglacial refugia hypothesis by examining the present-day distribution of *B. mucronatus* overlaid onto the pre-Pleistocene drainage pattern of the ancient Teays River Valley (Fig. 5). The distributions of all of the species within *Batrurur*, with the possible exception of *B. brachycaudus*, appear well correlated with pre-Pleistocene river drainage patterns rather than contemporary drainages (Fig. 5). The timing of speciation within *Batrurur* (Corrigan et al. 2014) also suggests that these species or their ancestors had well-established lineages during the Pliocene, when the Teays River flowed across what is now the Midwestern United States.

Two populations in Saline and Gallatin counties in southern Illinois occur south of the glacial limit (Figs 1 and 2, arrows D and E; Fig. 5). The presence of these populations, as well as the fact that non-karst populations are directly associated with glacial deposits, has been taken as evidence against *B. mucronatus* persisting in subglacial refugia during the Pleistocene (Holsinger 1981, 1986; Koenenmann and Holsinger 2001). It is possible, however, that these populations reflect more recent dispersal along the Wabash River into karst following glacial advances during the Pleistocene. This view is further supported by collections in Indiana along the lower Wabash River basin that were unavailable to Koenenmann and Holsinger (2001). Molecular studies presently underway should help elucidate the biogeography history of this species.

Batrurur brachycaudus appears able to occupy habitats similar to those where *B. mucronatus* is found, at least in central Illinois. However, nearly all populations of this species outside of central Illinois are associated with caves or karst springs (see Koenenmann and Holsinger 2001). One possible explanation is that the central Illinois populations formerly resided in solutionally enlarged conduits within carbonate rocks that were formerly exposed at the surface, or at least at the water table, during the Pliocene. Devonian limestones are present very near the surface in southern Champaign Co., Illinois (SSW of our new northeastern range extension for *B. brachycaudus*) and just to

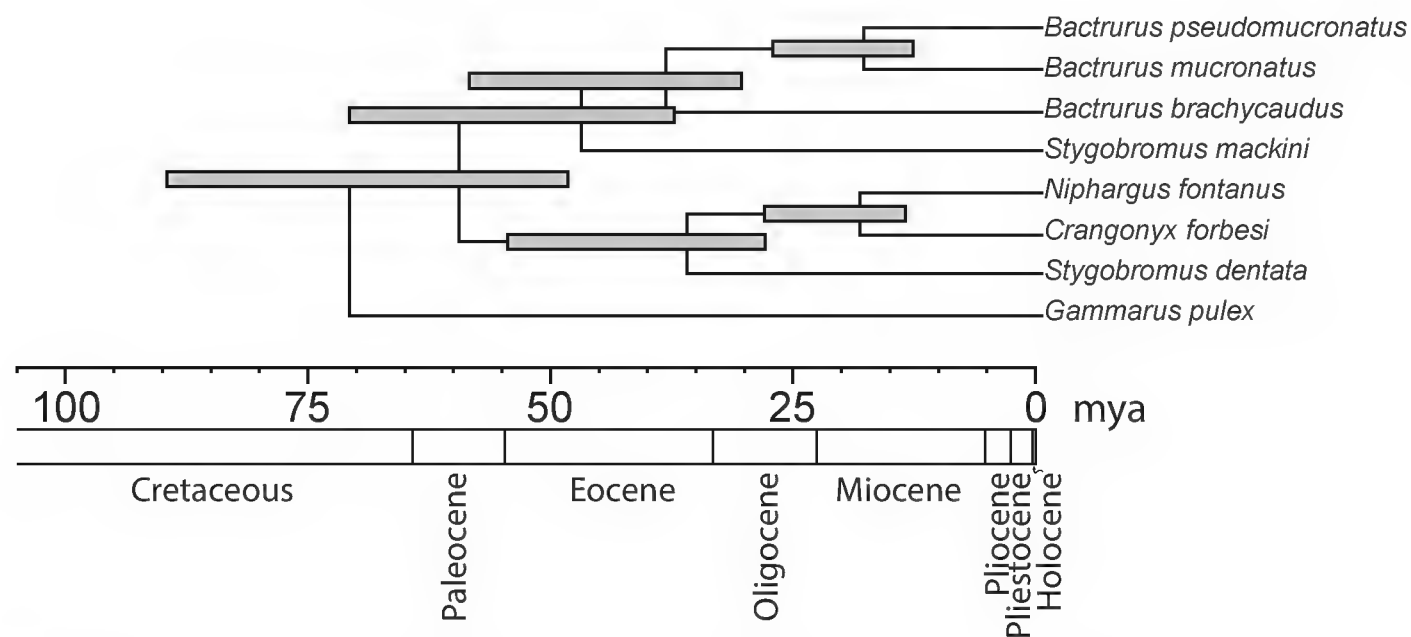


Figure 4. Groundwater amphipod relationships modified after the maximum clade credibility diagram of Corrigan et al. (2014) with 95% highest posterior density intervals shown as gray bars, and with additional details of geological timeline following Cohen et al. (2013).

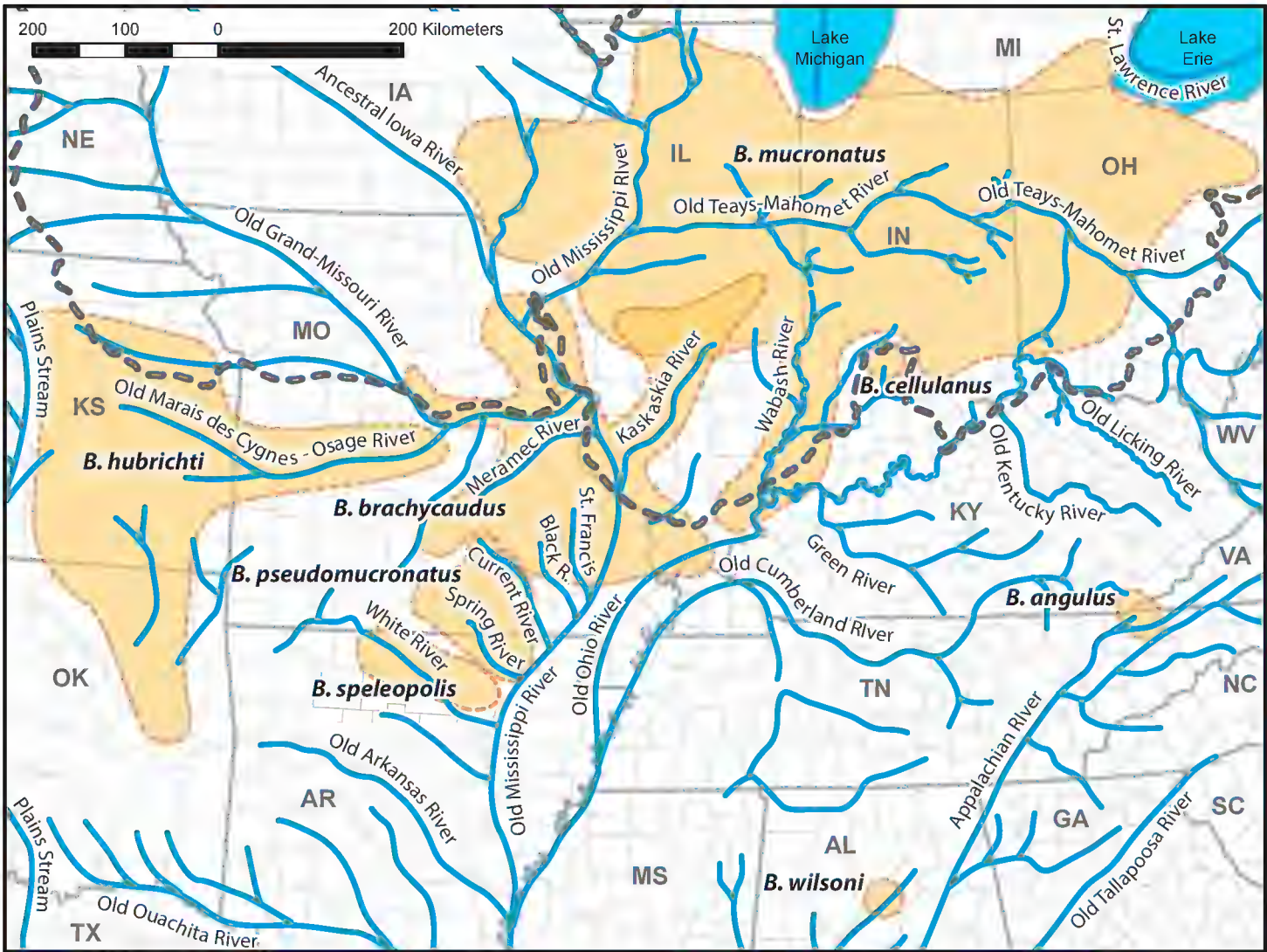


Figure 5. Species ranges for the genus *Batrurus* (Amphipoda, Crangonyctidae) in relation to pre-Pleistocene rivers of the mid-western United States. Shaded areas with thin dashed lines represent species ranges. For *Batrurus brachycaudus*, *Batrurus hubrichti* and *Batrurus mucronatus*, the range boundary has been interpreted in light of these rivers. Heavy dashed line is maximum extent of Pleistocene glaciation.

the south in Douglas Co., Illinois. Similarly, glacial deposits in Montgomery Co., Illinois, where one of the other two vexing localities of this species is located (Figs 1 and 2, arrow C), is underlain by carbonates of the Bond Formation (Willman et al. 1975, p. 196), which would have been exposed along river valleys during the Pliocene, when the Teays River was flowing westward through what is now central Illinois. It seems plausible, then, that this species, in addition to *B. mucronatus*, may have persisted throughout the Pleistocene in subglacial refugia.

The single Missouri population of *B. hubrichti* (Figs 1 and 2, arrow F) is interesting because it lies deep within the relatively continuous karst of the Missouri and Arkansas Ozark Plateaus developed in Ordovician dolomite. We confirmed the continued presence of this species from Miller Co., Missouri, where it had not been observed in nearly 75 years — since L. Hubricht collected seven specimens in August 1940 (Koenemann and Holsinger 2001). Remaining populations of *B. hubrichti* are associated with caves, springs, seeps, and wells in small disjunct carbonate outcrops, primarily in younger rocks of the Lower Permian Barnestone Formation of northeastern Oklahoma and eastern Kansas (Fig. 1). However, pre-Pleistocene river drainage patterns also may best explain the distribution of *B. hubrichti*, where the present-day distribution corresponds well with the old Marais des Cygnes and contemporary Osage rivers (Fig. 5). Other Ozark *Batrurus* species have distribution patterns that match both current and Pliocene river drainage basins: *B. speleopolis* is found in the White River and Curia Creek watersheds in Arkansas, *B. pseudomucronatus* is found in the Spring, Eleven Point, and Current river watersheds (Fig. 5). These two species have non-overlapping ranges and also do not co-occur with the more widespread *B. brachycaudus*. *Batrurus brachycaudus* presents a somewhat more confusing picture, but is associated with the Black River, St. Francis River, Meramec River and middle Mississippi River watersheds, perhaps extending up the Kaskaskia River watershed into central Illinois (Fig. 5).

Conservation assessments

Our conservation assessments indicate that five of the eight species of *Batrurus* are at an elevated risk of extinction under IUCN Red List or NatureServe criteria (Table 1), with one species (*B. cellulanus*) already extinct. Three species (*B. angulus*, *B. speleopolis*, and *B. wilsoni*) are ranked as “Critically Imperiled” (G1) under NatureServe criteria primarily because of very restricted distributions and very few number of occurrences. IUCN Red List assessments of these three species also reveal that each species is threatened, however, *B. speleopolis* and *B. wilsoni* were ranked as “Vulnerable” under criterion D2 rather than at higher risk category. This is because although both species have very restricted distributions and are known from very few occurrences, there is no current evidence for continuing, observed, inferred, or projected decline in EOO, AOO, quality of habitat, or number of occurrences or subpopulations. Lack of evidence for declines in these factors also explains differences in threat categories between Nature-

Serve and IUCN Red List assessments for *B. pseudomucronatus* and *B. hubrichti*. Both species were assessed as “Vulnerable” (G3) under NatureServe criteria but were assessed at lower risk categories under IUCN Red List criteria. *Batrachus brachycaudus* and *B. mucronatus* are two species at lowest risk of extinction according to both NatureServe and IUCN Red List assessments. Although populations of these species are not free from threats, both species are known from >100 occurrences, and there is no evidence that EOO or AOO has significantly declined.

Our NatureServe conservation ranks were generally very similar to previous assessments (Table 1; NatureServe 2015). *Batrachus wilsoni* was previously assessed as “Imperiled-Critically Imperiled” (G1G2), but we downgraded the status rank to “Critically Imperiled” (G1) because inventory efforts of caves and springs in northern and central Alabama since 2002 have not resulted in the documentation of additional populations. *Batrachus pseudomucronatus* was previously assessed as “Imperiled-Vulnerable” (G2G3). We upgraded the status rank to “Vulnerable” (G3) because of lower overall threat impacts, as most localities of this species occur on protected lands in rural areas that are predominantly forested. We downgraded the status rank of *B. hubrichti* from “Apparently Secure” (G4) to “Vulnerable” (G3). Although *B. hubrichti* has a large EOO, its distribution is likely fragmented, given the discontinuous extent of karst this species inhabits (Fig. 1). In addition, this species is still known from just 15 occurrences, as additional populations have not been reported in several years. Calculated status ranks were the same as previous assessments for all other species.

Threats

The most significant threat to all *Batrachus* species is groundwater pollution, but the sources, scope, and potential severity of groundwater pollution varies among species and also among populations within a species. Impacts from groundwater pollution can be chronic, occurring over years to decades, or acute, on the order of hours or days. Common sources of groundwater pollution include septic system leachate, sewage, urban and storm water runoff, livestock waste, and pesticides and other chemicals used in agriculture and residential areas (lawns and landscaping). Termiticide treatment on the grounds around Jordan Hall on the campus of Indiana University in Bloomington, Indiana, in the 1960s resulted in the extirpation of all groundwater life at the type locality of *B. cellulanus* (Koenemann and Holsinger 2001; Lewis 2012, 2015). Populations associated with karst are particularly vulnerable to groundwater pollution, as karst aquifers often have low potential for auto-depuration and have a high probability of retention of contaminants (White 1988; Kačaroğlu 1999; Ford and Williams 2007).

Conversion of land for development, agriculture, and logging can lead to increased sedimentation and changes in local hydrology, which may degrade the quality of habitat or reduce the amount of habitat available. Additionally, groundwater extraction for various human needs can result in reduction or complete loss of amphipod habitat. An increasing threat to populations of *Batrachus* in urban areas is the conversion of

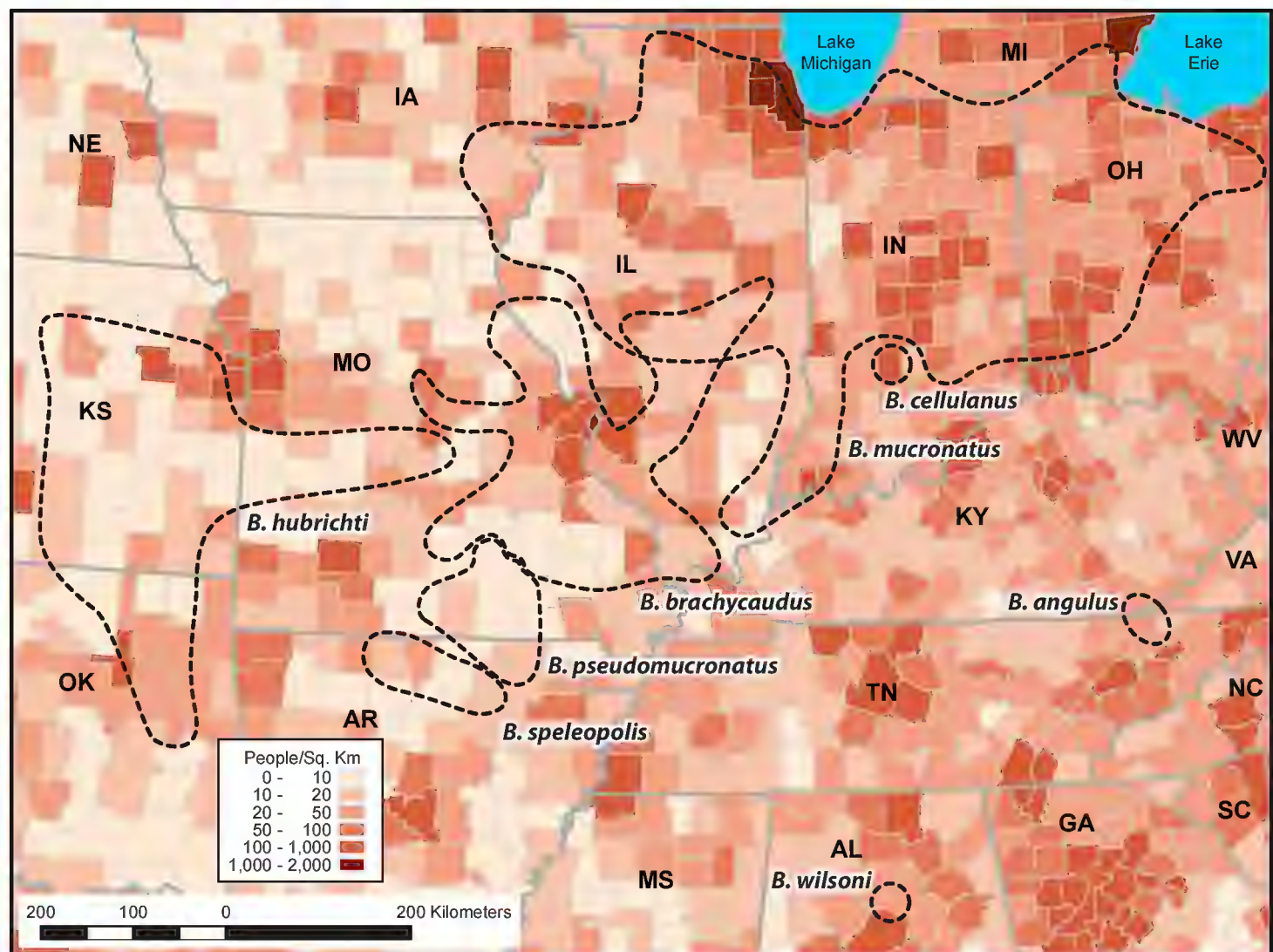


Figure 6. Human population density (2010 U.S. Census from the U.S. Census Bureau) by county with ranges for the eight *Batrachus* species. For *Batrachus brachycaudus*, *B. hubrichti*, and *B. mucronatus*, the range boundary has been interpreted as in Fig. 5.

land into impervious surfaces, such as roads, parking lots, and sidewalks. Impervious surfaces increase the speed and amount of storm water runoff leading to degradation of aquatic habitat through more rapid transport of contaminants and increased sediment load (Booth and Jackson 1997). Some populations of *B. brachycaudus* in the St. Louis, Missouri, metropolitan area and *B. mucronatus* in the Indianapolis, Indiana, metropolitan area occur where a significant (>30%) of land cover surrounding these populations has been converted to impervious surfaces, and these areas typically have high human population densities (Fig. 6).

Mining, natural gas, and oil drilling have impacted some populations of *Batrachus*. At least one population of *B. brachycaudus* was extirpated due to complete removal of a cave system by limestone mining operations in Ste. Genevieve Co., Missouri. A population of *B. mucronatus* is known from a reclaimed limestone mine in La Salle Co., Illinois, but this population wasn't discovered until after mining operations ceased. Coal strip-mining potentially impacts populations of *B. mucronatus* in southwestern Indiana (Lewis 2015).

Over-collection for scientific purposes can reduce or possibly extirpate local populations. However, there is no evidence to suggest that any populations have been extirpated

directly or indirectly due to scientific collection. Habitat disturbance caused by recreational cavers may pose a threat to some cave populations. *Batrachoseps* are typically found underneath rocks or within interstices in gravel and cobble in pools and shallow streams in caves. Consequently, there is a risk of mortality caused by trampling with increased cave visitation, but data are lacking regarding whether increased recreational caving significantly impacts *Batrachoseps* populations. Moreover, it is unclear whether most cave occurrences represent source or sink subpopulations. Source subpopulations of *Batrachoseps* species primarily associated with karst may occur in epikarst, the zone below the soil layer but perched above caves where water percolates from the surface into caves through pores, joints, and fissures. Epikarst has been poorly sampled and studied, and underappreciated as important habitat for subterranean species until recently (Culver and Pipan 2014). At least 38 species of the crangonyctid genus *Stygobromus* are associated with epikarst in the United States (Culver et al. 2010; Culver and Pipan 2014).

Climate change is predicted to have significant impacts on the levels, quality, and sustainability of groundwater (Taylor et al. 2012; Treidel et al. 2012; Klove et al. 2014). Climate change is expected to impose changes in several important environmental variables that directly influence groundwater organisms and ecosystem processes (reviewed in Klove et al. 2014), including changes in water temperature, dissolved oxygen, recharge rates, altered hydrological regime, and groundwater levels, as well as groundwater quality (Earman and Dettinger 2011; Treidel et al. 2012). Many subterranean species may be particularly vulnerable to impacts of climate change, because of their unique habitat requirements, endemism, adaptations, and often limited dispersal abilities. However, gaps in our understanding of distribution of groundwater organisms and groundwater ecosystem dynamics and services impair our ability to predict and manage species' responses to climate change.

Recommendations

Given results of our conservation assessments and available information on threats to populations, we offer several recommendations for study and management. First, studies are greatly needed to better ascertain the physical, chemical, and biological habitat variables that influence the survival of each species. These data are needed to better inform models of species distributions and responses to land use and climate change. Life history and demographic information are lacking for all *Batrachoseps* species. Research is greatly needed to determine population sizes, generation time, reproductive cycles, life span, fecundity, sex ratios, and survivorship. Such data are needed to quantitatively predict the future status of populations and species (e.g., population viability analysis). In addition, little information exists on diet, diseases, parasites, and other basic life history traits. Such information would be useful in the management of individual species and groundwater ecosystems. Future efforts should focus on locating additional populations of *B. angulus*, *B. speleopolis*, and *B. wilsoni*. Inventory efforts may be aided by the development of species distribution models to predict where each species may potentially occur.

Groundwater recharge zones and flow patterns should be delineated for populations of species of greatest conservation concern (i.e., *B. angulus*, *B. speleopolis*, and *B. wilsoni*). Vulnerability mapping can then be conducted to estimate the risk and impacts of groundwater pollution to aid in land management decisions and protection of sensitive groundwater species. Studies are needed to determine the sources, nature, and extent of local threats to significant populations. Water quality should be regularly assessed at select sites to monitor for possible changes that might negatively impact populations. Recent molecular studies have discovered high levels of cryptic diversity within widely distributed stygobiotic morphospecies (Finston et al. 2007; Zaksek et al. 2009; Niemiller et al. 2012; Eme et al. 2013), which has important conservation and management implications (Niemiller et al. 2013a). Phylogeographic and species delimitation studies should be a priority to ascertain if any of the three widely distributed species (*B. brachycaudus*, *B. hubrichti*, and *B. muconatus*) are actually comprised of multiple morphologically cryptic, genetically distinct lineages. Finally, it is somewhat surprising that *B. angulus* (in Tennessee), *B. speleopolis*, and *B. wilsoni* are not included on state endangered and threatened species lists, as all three species were assessed as threatened under both NatureServe and IUCN Red List criteria. We recommend that the conservation status of these species be reevaluated in their respective states in light of our conservation assessments.

Conclusions

Subterranean amphipods in the genus *Batrachus* are excellent candidates for addressing important questions in subterranean biogeography, as species in this genus occur in karst and non-karst habitats in both previously glaciated and non-glaciated regions in the central and eastern United States. Three species have extremely restricted distributions and are endemic to isolated karst areas. In contrast, three other species have exceptionally large distributions for subterranean fauna, which raises an important question of whether these species are presumably good dispersers or consist of assemblages of cryptic species with much smaller distributions. We suggest that the current distribution of *Batrachus* has been more influenced by drainage patterns and other events dating before the Pleistocene than to post-glacial dispersal. Five of eight *Batrachus* species were found to be at an elevated risk of extinction under IUCN Red List or NatureServe criteria, with one species already extinct (*B. cellulanus*). We identified 17 threats that either currently or may affect populations of *Batrachus* in the near future. Groundwater pollution represents the most insidious threat to all *Batrachus* species, although the sources, scope, and potential severity vary among species. Climate change may impact *Batrachus* and other groundwater species in the coming decades, particularly endemic taxa with small distributions, such as *B. angulus* and *B. wilsoni*. However, knowledge gaps in our understanding of distributions of groundwater taxa and ecosystem dynamics impair our ability to model and effectively manage species' responses to climate change and other threats. Research is also needed to better understand the

life history, ecology, and demography of *Batrachoseps*. Nonetheless, our conservation assessments strongly suggest that several species warrant consideration for state or federal listing as protected species and highlight the need for new conservation assessments (in the case of IUCN Red List) or reassessments (in the case of NatureServe) for groundwater and other subterranean species.

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